Bispecific T Cell Engagers (TCEs) are effective at inducing remissions in hematologic tumors, but their use in solid tumors has been challenging due to their extreme potency and unforgiving toxicity toward target expression in healthy tissue. To address this challenge, Amunix has developed conditionally active TCEs. XPATs or XTE Nyalted Protease-Activated bispecific T Cell Engagers targeting HER2 and EGFR that exploit the dysregulated protease activity present in tumors vs. healthy tissues, enabling expansion of the therapeutic index. The XPAT core consists of 2 single chain antibody fragments (scFvs) targeting CD3 and the tumor target. Two unstructured polypeptide masks (XTEN) are attached to the core that sterically reduce target engagement and extend protein half-life. Protease cleavage sites at the base of the XTEN masks enable protease-activation of XPAT in the tumor microenvironment, unleashing a small, highly potent TCE. In healthy tissues, where protease activity is tightly regulated, XPATs should remain predominantly inactive as intact prodrugs. In addition to localized activation, the short half-life of the unmasked PAT form should further widen the therapeutic index while providing the potency of T-cell immunity to improve the eradication of solid tumors.

XPAT PLATFORM

XPATs Are XTE Nyalted Protease-Activated T Cell Engagers

XPATs Enable Localized Tumor Killing, Limiting Toxicity Against Healthy Tissue Expressing the Target Antigen

RESULTS

Figure 1. XTEN Polypeptide Masks on HER2-XPAT Significantly Reduce T Cell–Mediated Cytotoxicity and T Cell Activation in vitro

A. Tumor-directed Cytotoxicity

B. Target-independent T cell activation

Figure 2. HER2-XPAT Induces Robust Tumor Regressions in Mice

A. Comparative Efficiency Induced with Equivalent Dosing of HER2-XPAT and HER2-PAT in NHPs

B. Efficiency in Depressing Prostrate Cleavage

Figure 3. XTEN Masks Significantly Expand Safety Margin of HER2-XPAT vs. PAT in Cynomolgous Monkeys

A. Maximal Tolerated Dose for HER2-XPAT vs. 40 mg/kg MTD for HER2-PAT

B. Tumor Cell Cytotoxicity

Figure 4. HER2-XPAT Induces T Cell Margination at Doses >2.5 mpk

A. Comparable Tumor Clearing in NHPs

B. ENP+ T Cells

C. Blood/Lymphocyte counts decline rapidly at 24 hours post dose, consistent with T cell activation

Figure 5. HER2-XPAT Fails to Activate Peripheral T Cells or Induce Cytokine Release Syndrome in NHPs, Even at 50 mg/kg Doses; HER2-PAT Induces CRS at 0.3 mg/kg

A. T Cell Activation (XPAT)

B. Peak Plasma Cytokines by Dose

Figure 6. XPATs Can Significantly Expand the Therapeutic Index of TCEs Directed Against Broadly Expressed Targets: EGFR

A. HER2-XPAT Provides ~100-fold Higher Tolerated Cmax than EGFR-PAT in NHPs

B. Summary/Conclusions

- In vivo, protolytically-unmasked HER2- and EGFR-XPATs demonstrate potent cytotoxicity against tumor lines with EOC50s in the single-digit pM range
- XTEN masking reduces target-directed T cell cytotoxicity and T cell activation by up to 15,000-fold
- In established xenograft models, HER2 and EGFR XPATs induced protease-dependent tumor regression with efficacious doses within an order of magnitude of the unmasked (active) T cell engager
- In cynomolgus monkeys, masked XPATs demonstrated significantly reduced CRS and increased tolerated exposures relative to unmasked PAT, suggesting favorable therapeutic index even for targets as broadly expressed as EGFR
- HER2- and EGFR-XPATs had maximum tolerated exposures that were >100-fold and >100-fold higher than those of their respective active forms (PAT)
- XPATs represent a novel strategy to improve the toxicity profile of T cell engagers while maintaining their potency against solid tumors, thus enabling a significant increase in the therapeutic index and expansion of target landscape for this potent modality